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NUTRITION REVIEWS

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Essential Fatty Acids, by Ralph T. Holman 33

Clinical Nutrition

Follow-Up Studies on Kwashiorkor 36
New Hypotheses Concerning Wilson's Disease 37
Urinary Amino Acid Excretion in Disease 39
Cholesterol in Diet and Serum 42
Thiamine in Human Milk 44
Amino Acid Supplementation of Rice 46
Hereditary Glycinuria 48

Animal Nutrition

Lysine and Feather Pigmentation 50

Experimental Nutrition

Measurement of Energy Content of Foodstuffs 51
Passage of Iodine Through the Placenta 52
Food Intake and Water Relationships 53
Metabolism of Essential Fatty Acids 55
Nutritive Value of Proteins 56
Feeding Patterns in Mice 58
Trans-Acids as Essential Fatty Acid Antagonists 59
Glucagon Effect on Gastric Activity 60

Notes

Letter to the Editor 62
American Board of Nutrition 62
Third Inter-African Conference on Nutrition 63
Recent Nutrition Books 64

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NUTRITION REVIEWS

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ESSENTIAL FATTY ACIDS

The importance of certain fatty acids was demonstrated in 1929 by the work of Burr and Burr (*J. Biol. Chem.* **82,** *345 (1929)*). Their work, and that of subsequent investigators, showed that the rigid exclusion of fat from the diet of rats induced cessation of growth, scaliness of skin, kidney damage, and impaired reproduction. All of these abnormalities can be prevented or cured by feeding oral supplements of less than 100 mg. linoleic or arachidonic acids per day per rat. Early reports suggested that linolenic acid was equivalent to linoleic in this respect, but more careful experimentation with purified linolenic acid has revealed that this substance stimulates the growth of fat-deficient animals, but that it is unable to cure the dermal symptoms of the deficiency.

Most of the experimentation on essential fatty acids has been done with rats. The studies have been extended to include mice, guinea pigs, rabbits, dogs, cattle, swine, and even insects. The wide range of animals which has been shown to require essential fatty acids suggests that the requirement is very general. Most species respond to the deficiency by a histological modification of the skin. The deficiency is more easily induced in young animals than in adult animals, and the requirement for essential fatty acids is greater in males than in females. For a detailed treatment of essential fatty acids and the deficiency thereof, the reader is referred to a recent review (*The Vitamins*, ed. Sebrell and Harris, *Academic Press, Vol. II, pp. 267–319 (1954)*).

The term "essential fatty acids" has been used in more than one sense in the scientific literature. In its broadest sense, it has been used to mean all the polyunsaturated fatty acids. *Properly, the term essential fatty acids should include only those substances which are active both for growth and for maintenance of dermal integrity, limiting the term to linoleic and arachidonic acids and to such other acids as may be derived metabolically from them.* In the interest of clarity one should use the specific chemical names of the substances whenever possible.

The common polyunsaturated acids are all straight chain fatty acids having two to six double bonds separated by single $-CH_2-$ groups, and having from 16 to 22 carbon atoms. The most important acids of the group are: linoleic acid, $CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7COOH$; linolenic acid, $CH_3CH_2CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_7COOH$; and arachidonic acid, $CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_3COOH$.

All the common polyunsaturated acids appear to be related to, or derived from, linolenic, linoleic or oleic acids, and they fall into three groups or families depending upon the structures of their molecules. Those related to linolenic acid have the first double bond between the third and fourth carbon atoms, counting from the terminal methyl group. The substances of this family which have been tested do not maintain dermal integrity, but they stimulate growth. Fatty acids related to linoleic acid (i.e., arachidonic acid) have the first double bond after the sixth carbon atom counting from the hydrocarbon end of the molecule, and these maintain dermal integrity as well as stimulating growth. Acids related to oleic acid have the first double bond after the ninth carbon atom and are presumably synthesized by animals, whereas members of the other two families cannot be synthesized, or cannot be synthesized in sufficient quantities, unless a dietary source of their precursors is furnished. It appears that animals are able to introduce additional double bonds in unsaturated acids between

the carboxyl group and the existing double bonds, but are unable to introduce new double bonds between the existing unsaturation and the methyl group at the end of the molecule. For example, it has been shown that arachidonic acid is synthesized from linoleic acid. Members of the individual families thus differ in the lengths of their carbon chains and in the number of their double bonds, but they have similar tail structures. It is also clear that the polyunsaturated acids are not all "essential fatty acids" in the same sense, and perhaps each individual acid or family of acids may function differently in animal metabolism.

Essential fatty acids are required for maintenance of a variety of biological functions. Deficiency results in diminished growth and structural changes in many tissues. The histological changes in the skin of deficient animals are accompanied by markedly increased water permeability. Kidney structure may become so deranged that hematuria results. Essential fatty acid deficiency leads to impaired reproduction. Pregnant female rats fed diets deficient in essential fatty acids do not deliver normal offspring, and degeneration of germinal epithelium has been observed in testes of male rats fed a diet which severely depleted them of essential fatty acids. The lipids of the gonads contain an unusually high proportion of polyunsaturated acids, suggesting the importance of these substances in reproductive function. The polyunsaturated acids are found in higher concentration in tissue structural lipids than in depot fats, and deficiency symptoms are most easily induced during physiological conditions when rapid cell division occurs.

Recently the essential fatty acids have been implicated in the problem of atherosclerosis. Although it is not known if hypercholesterolemia is a cause of atherosclerosis and/or myocardial infarction, it is considered to be an undesirable indicator of potential disease. The inclusion of high levels of saturated fat in the diets of animals and humans induces hypercholesterolemia, whereas diets containing a high level of unsaturated oils containing large proportions of essential fatty acids depress the level of plasma cholesterol (*Lancet* **II,** *943 (1957)*). The clotting time of blood seems to be prolonged and the fibrinolysin activity of blood is increased by the ingestion of fats rich in essential fatty acids, whereas diets rich in saturated fatty acids (hard fats) shorten clotting time and decrease the fibrinolysin activity. Therefore dietary essential fatty acids or polyunsaturated fatty acids exert three biological effects which tend to delay or prevent atheroma according to current concepts.

Recently lesions of the aorta of swine have been observed in swine fed a diet to induce essential fatty acid deficiency (*Proc. Soc. Exp. Biol. Med.* **95,** *274 (1957)*). The lesions induced were not atheromatous, but were found to be medial calcification. Treatment of depleted swine with concentrates of linoleic and linolenic acids caused relief of the medial calcification in proportion to the unsaturation of the acids fed (*Fed. Proc.* **16,** *232 (1957)*). Myocardial infarctions have recently been induced in adult rats by feeding them a diet which rapidly depletes them of essential fatty acids; that is, one which is deficient in essential fatty acids, and contains a high level of saturated fat, cholesterol and cholic acid (S. Hartroft, personal communication). A similar stressed essential fatty acid deficiency has been found to lead to lipid deposits in the coronary arteries of adult rats. These experiments used drastic conditions, but demonstrate that depletion of essential fatty acids may adversely affect the vascular system.

The relationship of essential fatty acids to cholesterol transport has been demonstrated recently (*Arch. Biochem. Biophys.* **52,** *180 (1954)*; *Ibid.* **57,** *520 (1955)*). Conditions which are accompanied by hypercholesterolemia, such as administration of dietary cholesterol, alloxan diabetes, or

hypothyroidism, were found to accelerate essential fatty acid deficiency in rats. We may postulate that if the composition of circulating lipids must remain reasonably constant, the need to transport large amounts of one component of the lipid mixture requires the animal to synthesize or release from his tissues matching quantities of the other components. Thus, any stress that causes increased transport of cholesterol or non-essential fatty acids, or the feeding of large quantities of these components, requires the release of polyunsaturated acids from tissues for incorporation in the cholesteryl esters and phospholipids which are constant components of the plasma lipid. If such a condition persists, the tissues may be depleted of essential fatty acids, the individual may exhibit symptoms of deficiency, and the composition of its plasma lipids may become abnormal. A corollary of this concept is that the requirement for essential fatty acids is relative to the amount of saturated fat in the diet. That is, the amount of essential fatty acids needed perhaps should be expressed as percentage of the dietary fat rather than as grams per day.

There has been a widespread feeling that essential fatty acids are not as important in human nutrition as they are in animal nutrition. Such a conclusion does not seem to be warranted in the face of present information. First, the general metabolic patterns are the same in animals and in humans, and one should not expect an exception in the case of the essential fatty acids. The inability to demonstrate dermal abnormalities in adult humans fed low fat diets is inconclusive, because the durations of the attempts were too short, the diets were not rigidly free of essential fatty acids, and an adult human has more than a pound of linoleic acid in his body. Moreover, essential fatty acid deficiency has been induced in adult animals only once, and then by very drastic conditions compared to those used to induce deficiency in weanling animals. On the other hand, Hansen and Wiese (*Fed. Proc.* **16,** *387 (1957)*) have demonstrated conclusively that essential fatty acids are required by the infant, for the skin lesions induced by feeding low-fat infant formulas responded to oral administration of linoleic acid. Although dermatitis has not been induced in adult humans by fat-free diets, changes in the dietary fat cause alterations in plasma lipids. For example, the feeding of a diet containing only saturated fat increases the ratio of trienoic to tetraenoic acids in human plasma. *Proc. Soc. Exp. Biol. Med.* **96,** *705 (1957)*). This is parallel to the observations that the tissues of animals deficient in essential fatty acids contain more trienoic acid than do normal tissues. These evidences suggest strongly that essential fatty acids are indeed required by humans.

Linoleic and linolenic acids, which are the precursors of the more highly unsaturated acids in animal tissues, are found in abundance in the common vegetable oils. The more highly unsaturated acids are found only in animal tissues. In muscle meats, the content of these is low compared to that of the non-essential fatty acids, but the fats of glandular tissues contain a much higher content of these acids. The fish oils also contain a high proportion of polyunsaturated acids. A diet containing a variety of vegetables, grains and meats should provide adequate quantities of essential and polyunsaturated acids. However, if it is true that essential fatty acid requirements are proportional to the intake of non-essential fatty acids, it would be wise to add to the concept of the balanced diet a consideration of the balance between essential and non-essential fatty acids in the dietary fat.

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FOLLOW-UP STUDIES ON KWASHIORKOR

The clinical picture of protein malnutrition, and the various means for its initial treatment, have become increasingly clear during the last two years (*Nutrition Reviews* **15,** *101, 132, 169, 199 (1957)*). Since this disease has been under active investigation for a relatively short period of time, data on long-term follow-up studies of children treated for kwashiorkor have been scanty. Consequently, a recent report by P. V. Suckling and J. A. H. Campbell (*J. Trop. Pediat.* **2,** *173 (1957)*) is a welcome addition to our knowledge of this disease.

Ninety instances of kwashiorkor were seen by Suckling (*M.D. Thesis Univ. London (1955)*) among negro children in Capetown, South Africa. Fifty of these were male infants. Thirty-one, including 13 males, died at the time of their original illness. Twenty-seven of the remaining 59 patients have been followed over a period of five years with one or more examinations. The mean age on admission was approximately 16 months and the subsequent examinations were made after periods of two and five years.

Initially these patients were similar to those accepted for metabolic study by J. F. Brock, *et al.* (*Lancet* **23,** *55 (1955)*). They were all edematous and hypoalbuminemic. Sixty per cent showed skin and mucous membrane changes and the liver was enlarged in 15 per cent. The follow-up examinations consisted of clinical assessment, determination of the hemoglobin concentration of the blood, and a liver biopsy. The social and economic conditions in the home were assessed by a medical social worker. The socio-economic status of the homes of the patients with kwashiorkor was poorer than a representative sample of the Cape negro population.

There was a low recurrence rate in this group. Only two of the 59 patients who recovered from their initial episode were re-admitted due to recurrence of the disease, both of these three months after discharge from hospital. The general health of the children was good at both the two-year and five-year follow-up examinations. One case of primary tuberculosis was found on each occasion. Although there was no long-term recurrence of symptoms suggesting kwashiorkor, five children had skin or mucous membrane changes apparently of nutritional origin at the five-year examination, and one child was found to have an enlarged liver with abnormal liver function tests but no abnormality was noted on the liver biopsy.

The average hemoglobin concentration at the time of the two-year follow-up was 11.3 g. per cent with a standard deviation of ± 1.34 g. per cent and was interpreted as a continuing mild anemia. At the time of the five-year follow-up, the average hemoglobin concentration was 13.5 with a standard deviation of ± 0.97 g. per cent, which was considered as normal. The height and weight were presented graphically. When compared with equivalent data for a similar population, the growth in height of the children recovered from kwashiorkor was parallel to, but slightly below, the norm. This difference was of only borderline statistical significance. The weight on admission averaged 12 pounds. During the first two years of follow-up there was a very rapid weight gain but the regression line for the two to five year period was not different from the norm.

Although liver biopsies were not performed in these children at the time of their original illness, there is ample reason, from subsequent studies of children from the same community, to expect that fatty infiltration would occur in 95 per cent of the cases. In striking contrast to the presumed background of abnormality, severe fatty changes were not seen in any specimen. In three instances fat was present in a modest amount. The features of cirrhosis were con-

sistently absent at both two years and five years following recovery. The results of this study indicate, therefore, that the liver can recover completely from the changes observed in kwashiorkor, and that the almost universal fatty infiltration is not necessarily followed by fibrosis.

While more long-term follow-up studies of the treatment of kwashiorkor are needed from representative areas throughout the world, it is apparent from this study in Capetown that recovery from protein malnutrition may be essentially complete in so far as it can be measured, and that this will occur during the first two years after therapy is initiated. The low relapse rate in this particular period may not be representative in that all the patients were referred to child welfare stations for further medical care.

NEW HYPOTHESES CONCERNING WILSON'S DISEASE

Wilson's disease is characterized by the presence of nodular cirrhosis, the development of basal ganglion and cerebellar dysfunction, and the presence of a brownish discoloration of the margin of the cornea (the Kayser-Fleischer ring, which is composed of deposits of copper and other trace metals). The biochemical abnormalities include the increased concentrations of tissue copper mentioned above, increased copper excretion in the urine, a decrease or absence of the copper binding protein of the plasma, ceruloplasmin, and the presence of increased quantities of amino acids and oligopeptides in the urine.

The important observations of J. N. Cummings (*Brain* **71,** *410 (1948)*), that the brain and liver of patients with Wilson's disease contain increased amounts of copper, have stimulated intensive investigation into the pathologic physiology of this condition. Despite the intense activity in this field, the ultimate mechanisms of this disease remain to be uncovered. Earlier research has been reviewed repeatedly in this journal (*Nutrition Reviews* **5,** *255 (1949)*; **12,** *198, 304 (1954)*; **13,** *232 (1955)*).

Speculations have been offered concerning the possible relationship between ceruloplasmin deficiency and the mechanism of the disease since I. H. Scheinberg and D. Gitlin described this biochemical abnormality in 1952 (*Science* **116,** *484 (1952)*). This protein is a minor component of the alpha globulins and has been named because of its characteristic blue color. It has a molecular weight of 150,000 and eight copper atoms are bound to each protein molecule. The firmness of this binding has made it difficult to attribute a specific transport or regulatory function to ceruloplasmin.

The nature of this binding has been examined recently by I. H. Scheinberg and A. G. Morell (*J. Clin. Invest.* **36,** *1193 (1957)*) using the radioactive isotope of copper, Cu^{64}. Little exchange of ceruloplasmin copper occurs when it is equilibrated with ionic Cu^{64} in the presence of a simple buffer. The situation is quite different when ascorbic acid was added to the *in vitro* system. Ascorbic acid is a substrate for the enzymatic-like action of ceruloplasmin. In its presence ceruloplasmin became colorless and it could be demonstrated that at least half of the copper atoms of ceruloplasmin were readily exchangeable. The demonstration of the reversible nature of copper binding suggested to the authors that ceruloplasmin itself might be a regulator of copper absorption. The normal absorption of copper from the gastrointestinal membrane is probably dependent on a concentration gradient. If conditions in the intestinal plasma promoted the release of ceruloplasmin copper this might build a local increase in ionic copper concentration which would inhibit absorption. In Wilson's disease this brake would be absent. This

ingenious suggestion far outstrips the presented data because the authors have only demonstrated exchange and not a significant increase in free copper concentration under *in vitro* conditions. The possible unique conditions of intestinal plasma which would promote copper release remain speculative.

One of the features of Wilson's disease which has intrigued investigators concerns the possible relationship between the coppruria and the aminoaciduria. It has been suggested that the increased copper in the urine was a result of chelation of the copper by the amino acids or peptides in the urine. This feature of the disease has been scrutinized by F. L. Iber, T. C. Chalmers and L. L. Uzman (*Metabolism* **6**, *388 (1957)*). First of all, they measured the urinary excretion of copper following the oral administration of 10 to 20 g. doses of the following amino acids to a patient with Wilson's disease: D,L methionine, L-arginine, D-alanine, glycine and L-glutamic acids. Only after the administration of methionine was there a significant increase in urinary copper over the base line level. Their second observations concerned the effect of increasing the protein intake from 10 to 110 g. per day on the urinary excretion of copper and amino acids. As reported by others there was an immediate increase in the excretion of copper and amino acids in the urine on the higher protein intake. This increase in copper excretion and the rise of urinary amino acids did not persist after the first three days of the augmented protein intake. A similar response was observed when casein was substituted for the mixed proteins during the high protein period.

Although the observations made by Iber and co-workers are not strikingly different from previous experience, the interpretation of the findings by the authors is of interest. They argue that there is not a direct relation between the aminoaciduria and the coppruria. In the first place, certain patients show a poor correlation between coppruria and aminoaciduria. Secondly, aminoaciduria induced in normal subjects does not provoke coppruria. Thirdly, chelation of copper probably occurs with oligopeptides and not with amino acids. Finally, the administration of single amino acids did not, with the exception of methionine, induce coppruria in these experiments. It was suggested that the rise in copper excretion, which occurred with a sudden increase in dietary protein, was due to the release of intracellular labile copper by a "washing out" process which is effected by oligopeptides and not so much by amino acids. The decrease in the excretion of copper which occurs with continued high protein intake was attributed to depletion of the intracellular labile copper pool. A similar response occurs with BAL. The increased coppruria following methionine was attributed to stimulated intracellular protein turnover. The hypothesis of Iber and co-workers is not established by the sketchy data available. In order to distinguish renal or peripheral action of protein and amino acids in promoting coppruria, direct data are needed concerning the renal clearance of the copper fractions in the presence and absence of aminoaciduria. Perfection of existing methods should make direct measurements possible.

The two papers which have been reviewed present fresh hypotheses concerning the metabolism of copper in Wilson's disease and both hypotheses require more direct evidence before they can be generally accepted. The elucidation of the elusive mechanisms of this disease will not be a simple matter and remains a challenge to investigators.

URINARY AMINO ACID EXCRETION IN DISEASE

Plasma or urinary amino acid levels have been studied in a variety of diseases and conditions; after burns or surgery (*Nutrition Reviews* **12,** *328 (1954)*), after administration of protein hydrolysates (*Ibid.* **7,** *68 (1949)*), and during infectious hepatitis (*Ibid.* **13,** *266 (1955)*). Massive liver necrosis has long been known to cause a generalized increase in plasma and urine amino acids, with precipitation of tyrosine and leucine crystals in the kidney tubules and urine.

Various other examples of abnormal, and often heavy, amino acid excretion are discussed below. A number are genetically determined, and the evidence indicates that the abnormality often lies in renal tubular reabsorption. H. E. Harrison and H. C. Harrison (*J. Am. Med. Assn.* **164,** *1571 (1957)*) have summarized the information on most of these conditions in convenient reference form, with numerous bar charts, giving the amounts excreted of each of 23 amino acids in each disease.

Some work has been done on the renal clearance of individual amino acids in man (*Nutrition Reviews* **14,** *139 (1956)*); wide variations exist. The work is tedious and the findings remain incomplete.

Studies on cystinuria, long classified as an "inborn error of metabolism", show that large amounts of the basic amino acids, lysine, arginine, and ornithine, are also excreted in the urine, apparently throughout life, in this familial disorder. Cystine, although not the most abundant of the four, is the least soluble, accounting for the development of the cystine calculi which are characteristically seen. H. Harris and E. B. Robson (*Am. J. Med.* **22,** *774 (1957)*) have given an excellent account of the present knowledge of cystinuria. The theory that this is a disorder of intermediary metabolism of cystine appears invalidated by the finding that four amino acids are excreted, that there is no accumulation of cystine in the plasma, and that renal clearances of all four are much increased over the normal (as high as thirty times in the case of cystine). The evidence is that the disease is a defect of tubular reabsorption (*Nutrition Reviews* **13,** *198 (1955)*). Cystine is also excreted in excessive amounts in Fanconi's syndrome, Wilson's disease (hepatolenticular degeneration) and cystinosis.

Harris and Robson point out that cystinosis may be sharply distinguished from cystinuria on clinical, biochemical, and genetic grounds; ten or more amino acids are excreted in the former, and stone formation is not a feature.

Histidinuria is apparently a normal phenomenon in pregnancy. Little is known of its mechanism.

In phenylketonuria or phenylpyruvic oligophrenia, another enzymic defect of metabolism, there are excreted a gram or more per day of phenylalanine and comparable amounts of related compounds. This genetic disease, a recessive condition characterized by mental deficiency and the excretion of phenylpyruvate, is essentially a result of failure to oxidize phenylalanine to tyrosine, due to absence or inactivity of the appropriate liver enzyme. The subject is comprehensively reviewed by W. E. Knox and D. Y.-Y. Hsia (*Am. J. Med.* **22,** *687 (1957)*). There is no reason to believe a renal defect exists.

Perhaps the most striking example of excessive excretion of amino acids is afforded by patients with the syndrome described at different times by de Toni, Debré and Fanconi (*Jahrb. f. Kinderh.* **147,** *299 (1936)*). This congenital and familial disease is associated with glycosuria, heavy aminoaciduria (without changes in the corresponding plasma levels), and phosphaturia. These lead to the retardation of growth and rickets seen in children and to the osteomalacia and pseudofractures which eventually incapacitate adults, and have been shown to

be caused by diminished renal tubular reabsorption of glucose, amino acids, and phosphate. It is not known whether the occurrence of cirrhosis is a primary lesion or secondary to amino acid loss.

One 26-month-old child with Fanconi's syndrome studied by S. E. Bradley (*Am. J. Med.* **20,** *448 (1956)*) had two to four plus proteinuria and excreted up to 8 g. of amino acids per day. Fifteen of the latter amino acids were distinguished by a method which showed only three in normal urine. Glomerular filtration rate and renal plasma flow were normal and the maximum glucose reabsorption (T_m) was above normal, suggesting that the mass of tubular tissue was not reduced. Microdissection and pathologic studies of the kidney have shown tubular changes in Fanconi's syndrome, though a distinctive lesion has not been recognized generally. Although there is good evidence that the proximal segment is the site of glucose reabsorption, that of amino acid reabsorption is not established; it may well be the same. There is no doubt that most of the manifestations of Fanconi's syndrome are due to altered renal tubular function. But whether the disease is renal or is a generalized disease of cell function, of which the tubular disturbance is but one manifestation, is not proven. The latter seems likely.

Because of the presence of cystinosis in Lignac's disease, the latter is considered an entity distinct from Fanconi's syndrome by most investigators. The term Lignac-Fanconi syndrome is used by some, however.

Most patients with Fanconi's syndrome die before puberty. L. A. Wallis and R. L. Engle, Jr., (*Ibid.* **22,** *13 (1957)*) have reviewed 18 recorded cases of the disease in adults. Aminoaciduria was present in all 13 instances in which it was measured; plasma levels were low or normal in all nine occurrences studied. In addition to hepatolenticular degeneration (Wilson's disease) the authors found isolated examples, in their search of the literature, of aminoaciduria associated with glycosuria occurring with large doses of vitamin D_2, glycogen storage disease, lead poisoning, a severe lysol burn early in the diuretic recovery phase, and experimental injection of maleic acid intraperitoneally. Many of these episodes proved reversible. One case had Fanconi's syndrome with multiple myeloma and inclusions of crystal-like material in renal tubular epithelium, together with the heavy casts which occur in cases of myeloma.

Because of the strong evidence linking Fanconi's syndrome to the renal tubule, and the fact that "renal tubular acidosis" is apparently often attributable to pyelonephritis, W. Lathem, K. Baker and S. E. Bradley (*Ibid.* **18,** *249 (1955)*) have studied "Urinary amino acid excretion in renal disease, with observations on the Fanconi syndrome". They did paper chromatograms on the urines of their subjects fasting and during infusion of a 5 per cent infusion of a partial fibrin hydrolysate. Thirteen of their subjects suffered from various well documented kidney diseases. The urinary amino acid patterns closely resembled the normal and differed from the heavy aminoaciduria found in one patient with Fanconi's syndrome in whom they demonstrated normal renal blood flow and filtration. Infusion of the fibrin hydrolysate in this patient did not show an altered excretory response recognizable from the paper chromatograms, suggesting that factors other than tubular damage may be involved. It would seem that quantitative studies with associated glucose excretion figures would be needed to resolve the matter, however.

Amino acid excretion has been studied in liver disease and Wilson's disease (*Nutrition Reviews* **8,** *252 (1950)*). A. G. Bearn (*Am. J. Med.* **22,** *747 (1957)*) has summarized our present knowledge of the latter, whose principal manifestations are those of familial cirrhosis and lenticular degeneration in the second decade of life. The basic inherited abnormality involves copper metabolism and is presumed to be a defective

synthesis of ceruloplasmin with low serum copper and increased albumin-bound copper. The reason for increased copper absorption from the gastro-intestinal tract is not apparent. At any rate, an increased amount accumulates in the liver, brain, and other tissues. Urinary copper excretion is excessive. Accumulation of copper in the kidney is believed responsible for the characteristic renal lesion which is similar in many ways to that seen in Fanconi's syndrome. Although the serum amino acid pattern is normal, there is often aminoaciduria (especially of threonine and cystine, both of which may be excreted in amounts up to 20 times the normal). There is phosphaturia, uricosuria is common, and glycosuria may occur with normal plasma levels. Although the evidence favors the view that the urinary changes are the result of copper damage to the renal tubules (*Nutrition Reviews* **12,** *304 (1954)*), others assume, on the contrary, that heavy copper deposition is secondary to the excessive excretion of amino acids and peptides and the low plasma ceruloplasmin levels (*Ibid.* **12,** *198 (1954)*).

Aminoaciduria has been noted in galactosemia, another inborn error of metabolism (*Ibid.* **14,** *5 (1956)*). This rare congenital disorder of carbohydrate metabolism manifests, in addition to galactosemia and galactosuria, mental retardation, cataracts and hepatomegaly. A. Holzel, G. M. Komrower and V. Schwartz (*Am. J. Med.* **22,** *703 (1957)*) have reviewed the subject, and point out that the galactosuria, proteinuria and aminoaciduria all disappear when lactose and galactose are removed from the diet. Chromatograms in galactosemia show a considerable increase in excretion of six amino acids and a lesser increase in that of four others. The great increase in α-amino nitrogen clearances in the presence of normal plasma levels and normal urea clearances indicates a renal mechanism. Competition between galactose and amino acids in tubular reabsorption or renal toxicity of galactose are possible mechanisms. However, although galactosuria is heavy within 24 hours of milk feeding, amino nitrogen excretion does not increase until the fifth or sixth day. With the demonstration that erythrocytes from galactosemic subjects lack the enzyme that converts galactose-1-phosphate to glucose-1-phosphate with resulting accumulation of the former, deficiency of the enzyme seems established as the essential feature of this hereditable disease (Schwarz, L. Golberg, Komrower, and Holzel, *Biochem. J.* **59,** *xxii (1955)*). The same deficiency has been shown in galactosemic liver, and it is reasonable to suppose it exists in all the cells of the body. Interestingly, it may well be that it is not galactose, but galactose-1-phosphate, which is the toxic substance.

In considering the various aminoacidurias reviewed above, one is struck by the fact that many are genetically determined, and that they are associated with defects in tubular reabsorption of glucose, phosphate, or of other amino acids. Harris and Robson, in the work cited above, point out that the lysine, arginine and ornithine losses in cystinuria imply one step in renal tubular reabsorption of these acids which is common and specific for the four, and defective in the disease cystinuria. They refer to work showing that raising the plasma lysine level of normal subjects leads to leakage of the other three, implying that a common reabsorptive mechanism in the tubule has been overloaded. Similar studies should be helpful in showing whether there is an important connection between the various substances excreted in, for example, Fanconi's syndrome.

Specifically, the relation of sugar to amino acid excretion is not clear. They are clinically associated in Fanconi's syndrome, Wilson's disease (occasionally), galactosemia and the isolated cases cited above. The same segment of tubular epithelium may absorb both, but since amino acids and glucose apparently form chemical combinations, this

may account for their associated loss in the urine. Intravenous infusions of protein hydrolysates were administered to a group of subjects by H. N. Christensen, P. B. Wilber, B. A. Coyne, and J. H. Fisher (*J. Clin. Invest.* **34,** *86 (1955)*). Addition of glucose to the mixture increased the excretion of amino acids and peptides slightly. But when the mixture was *autoclaved* with glucose, 5 per cent of the amino acids and 30 per cent of the peptides were lost in the urine. At present it is not clear how much of the association between glycosuria and aminoaciduria is anatomical, chemical, or neither.

It is clear that the urinary amino acid excretion pattern in man has been increasingly studied with the wider use of chromatographic methods, and that such studies will lead to a better understanding of amino acid metabolism, renal mechanisms, and possibly to laboratory diagnostic procedures.

CHOLESTEROL IN DIET AND SERUM

Many investigators are currently of the opinion that atherosclerosis is primarily the result of a disordered cholesterol metabolism. Evidence supporting this view has come from two principal lines of investigation: (1) Cholesterol has been found in high concentrations in the lesion; and (2) administration of cholesterol to the rabbit, the chicken, the hamster, and the guinea pig (L. N. Katz and J. Stamler, *Experimental Atherosclerosis, Charles C. Thomas and Co. Springfield (1953)*) and the thiouracil-treated dog (A. Steiner and F. E. Kendall, *Arch. Path.* **42,** *433 (1946)*) results in elevations of the blood cholesterol and in lesions of the arteries which resemble those observed in human atherosclerosis.

Although it is difficult at the present stage of investigation to measure the effect of cholesterol feeding on human atherosclerosis, A. Keys (*Fed. Proc.* **8,** *523 (1949)*; *Circulation* **5,** *115 (1952)*) and Keys and J. T. Anderson (*Nat. Research Council Pub.* **338** *(1954)*) have previously reported briefly both survey and experimental evidence indicating that the cholesterol in ordinary human diets has substantially no effect on the concentration of cholesterol in the blood. However, these authors have more recently collaborated with others (*Keys, et al., J. Nutrition* **54,** *39 (1956)*) in an extensive series of dietary experiments on human subjects under very rigidly controlled conditions, and also in both cross-sectional and longitudinal studies on men in Minnesota and Sardinia.

All of the subjects were men between the ages of 20 and 60 and were pronounced to be physically healthy on the basis of medical examinations, including electrocardiography. Subjects in the prolonged controlled experiments were schizophrenic patients in Hastings State Hospital, Minnesota. Men in the longitudinal surveys were professional and business men aged 45 to 55 in 1947 who had cooperated with the laboratory for at least nine years. Surveys on the island of Sardinia included coal miners in the small town of Bacu Abis and moderately active men employed by the city of Cagliari and by the University of Cagliari Medical School.

Dietary cholesterol was computed from food charts. In surveys, except for those where the diets were measured by a U. S. Department of Agriculture group, the dietary intake of cholesterol-containing foods was estimated by individual interviews, with particular attention being given to the intake of eggs, cream, butter, cheese, ice cream, meats, fish and chicken. On the island of Sardinia the individual habits of eating eggs most wholly accounted for the variation in total cholesterol intake. The

mean for a total of 187 men was 4.92 eggs per week with the range extending from zero to 30 eggs per week.

Results of six cross sectional surveys on men in Minnesota showed clearly that any relationship between serum cholesterol and that consumed in the diet must be very small. However, it appeared that there is a slight tendency for men who consume the least cholesterol to have concentrations of blood cholesterol slightly lower than the average while those who eat the most cholesterol have the opposite tendency, the deviations in each case being approximately 3 per cent of the mean. Complete independence prevailed over the middle range of intakes, including 60 per cent of the subjects.

However, the authors argue that these slight trends are not necessarily indicative of any effect of dietary cholesterol itself. In the first place the dietary cholesterol was expressed in absolute units and differences in these absolute amounts. Thus two men, differing both in body size and physical activity and therefore consuming different amounts of food when they are in caloric balance, will consume different total amounts of cholesterol even though the composition of the diet is exactly the same. A more serious question is whether the observed variations in cholesterol intake are independent of important differences in other characteristics of the diet composition. Indeed, these investigators found that cholesterol intake in the American diet was almost always related to the presence of animal protein and especially animal fat in the diet. It was noted that the cholesterol intake of men on high fat diets averaged 20 per cent more than for the men on low fat diets.

Among a large number of Minnesota business and professional men studied since 1947 there were 33 men whose jobs, diets, and body weights were very constant over a period of four years, and whose dietary intakes of cholesterol were consistently in the lower third of the distribution of the intakes of the entire group. The authors were able to contrast these men with 35 of their fellows similarly constant in work, diet and body weight, whose average cholesterol intake was in the upper third of the distribution. It was observed that the long-standing consumption of more or less dietary cholesterol produced no significant difference in the average serum cholesterol level between the two groups.

In this same population there were 64 men who made major changes in their dietary cholesterol but who otherwise maintained their dietary habits and who did not change in body weight by more than five pounds. When these men changed their cholesterol intakes, either up or down, there was no significant change in the serum level.

In the spring of 1953 and in 1954, the nutrition research staff from the Agricultural Research Service of the U. S. Department of Agriculture carried out a study of the diets consumed by 119 men in the same general group of business men mentioned above. Because of the possibility that different relationships would be observed with different levels of total fat in the diet, separate analyses were made for 61 men who consumed more than 40 per cent of their calories in the form of fats and 58 men who consumed less fat. In neither unit was there a correlation with the serum cholesterol concentration.

A modified rice-fruit diet was devised and given to psychotic patients. A daily allowance of 11 g. of fat was included, either in the form of egg yolks or in the form of all-vegetable oleomargarine so that the daily cholesterol diet was 500 to 600 mg., or zero. Serum cholesterol was measured at the end of four weeks of either diet. Caloric balance was maintained throughout. The change from regular to modified rice-fruit diet for four weeks produced a 15 to 20 per cent average drop in the blood serum cholesterol and

this was not significantly affected by the presence or absence of cholesterol. Change from the rice-fruit diet back to an ordinary diet produced greater increases than the preliminary decreases, averaging 20 to 30 per cent.

In a special study devised to eliminate the effects of variables other than the dietary cholesterol, a series of rotating menus was developed so that the daily nutrient intake varied little though the diet was not particularly monotonous. The mode of life of the subjects (schizophrenic men) was carefully standardized with respect to exercise and recreation. In one experiment, 13 men consumed 374 mg. of cholesterol daily for the first four weeks and then received an average of 1369 mg. daily for the final four weeks. In another experiment, 14 men went through the reverse order of change in cholesterol intake. There were suggestions of a trivial response of the serum cholesterol to the diet but in neither experiment was this statistically significant.

Results of the two surveys in Sardinia in 1955, one on moderately active municipal employees in Cagliari and one on poor coal miners in the small mining town of Bacu Abis (representing a two-fold difference in cholesterol intake) failed to show significant differences in serum cholesterol levels.

In another series of experiments and surveys carried out by Keys *et al.* (*Nutrition Reviews* **14,** *67 (1956)*) it was shown quite definitely that, although it is easy with different populations to point out an apparent relationship between cholesterol intake and concentration in the blood, other peculiarities correlated with average cholesterol intake seem actually to be responsible. One of the factors which seems to provoke the appearance of an unusually high concentration of cholesterol in the serum is the ingestion of large amounts of fatty acids, in various forms but especially as neutral fats.

The results of these studies support the view that endogenous synthesis of cholesterol is the primary factor in human regulation of the concentration of this substance in the blood. As indicated by various experiments in animals, the liver probably is inhibited to the proper degree in its synthesis of cholesterol when this is present in the circulation by virtue of the diet. As the authors state, it is probable that when cholesterol is artificially added to the diet in increasingly large amounts, a point will be reached where there will be a positive effect on the serum level in man.

THIAMINE IN HUMAN MILK

Unlike the constant level of B vitamins in cow's milk, the level of certain B vitamins in human milk, especially thiamine, is known to be influenced by the dietary intake of vitamins and stage of lactation (*see Nutrition Reviews* **1,** *243, 270 (1943)*; **4,** *134 (1946)*; **5,** *107 (1947)*; I. G. Macy, *Am. J. Dis. Child.* **78,** *589 (1949)*; S. K. Kon and E. H. Mawson, "Human Milk", *Medical Research Council Special Report, H. M. Stationery Office, London, No. 269 (1950)*; R. Chanda, E. C. Owin, and B. Cramond, *Brit. J. Nutrition* **5,** *228 (1951)*). Because of the incidence of infantile beriberi which still exists in certain areas of the world, information on factors affecting the thiamine content of human milk is important both from a theoretical and practical standpoint.

During an investigation on the prevalence of beriberi in Andhra State of South India (a rice consuming area), a study was made on factors affecting the thiamine content of human milk by B. V. Rama-Sastri and D. Indravati (*Indian J. Med. Res.* **45,** *447 (1957)*). A total of 126 samples of milk from mothers at various stages of lactation was studied. Thiamine was assayed by a modification of the methods of N. Kendall (*J.*

Pediat. **20,** *65 (1942)*) and Kon and Mawson (*loc. cit.*). Morning samples of milk were obtained from mothers chiefly representing a "poor class" residing in Visakhapatnam town and surrounding villages. A preliminary test with six women demonstrated that the thiamine content of milk remained constant during the course of emptying the breast so in subsequent sampling this was not considered as a variable.

In confirmation of other studies, the results of Rama-Sastri and Indravati showed that the thiamine content of milk in the early stages of lactation was very low, with an average of 2.6 micrograms per 100 ml. from one to two weeks after gestation. Following this there was a gradual increase in thiamine concentration until the second month of lactation when the values reached an average of 11.9 micrograms per 100 ml. After this period, and until the eighth month, the thiamine content rose only very slightly (but not significantly so). These values are slightly lower than those reported for milk from other areas of the world, particularly from England and America. They are also less than half the level of thiamine in cow's milk (*Nutrition Reviews* **1,** *243 (1943)*; **7,** *275 (1949)*).

When the subjects were divided by age and by number of children there were no significant differences in the thiamine content of the milk from the different groups. However, differences were observed when the milk of mothers from various localities was compared (ranging from 9.8 micrograms per 100 ml. in Visakhapatnam to 18.0 micrograms in Pendurti). This led the authors to study the effect, on the amount of the vitamin in milk, of changes in the thiamine content of the diet. This was done by giving tablets containing 10 mg. of thiamine hydrochloride to 18 mothers (beyond one month of lactation) daily for seven days and comparing the thiamine content of the milk before and after supplementation.

It was found that there was a significant rise in the thiamine content of the milk from mothers supplemented with thiamine. Increases were especially notable in milks which previously were unusually low in thiamine, but this was not true in all samples. The average values of milk from the 18 mothers increased from 10.0 micrograms to 15.6 micrograms per 100 ml. after thiamine supplementation of the diet. As pointed out by the authors, similar effects have been observed by others, including A. F. Morgan and E. G. Haynes (*J. Nutrition* **18,** *105 (1939)*; E. M. Knott, S. C. Kleiger, and F. Terres-Bracamonte, *Ibid.* **25,** *49 (1943)*; and J. P. Pratt and B. M. Hamil, *Ibid.* **44,** *141 (1951)*; *also see* Kon and Mawson, *loc. cit.* and Macy, *loc. cit.*).

The authors (Rama-Sastri and Indravati) refer to unpublished work at Coonoor Nutrition Research Laboratories which indicates that the average daily milk yield by mothers in "the poor and lower middle classes in South India" was only about 600 ml. Therefore, the average daily intake of thiamine of infants in this area would be less than 0.075 mg. per day. This is considerably less than the usual recommended levels of 0.14 to 0.20 mg. per day (*Nutrition Reviews* **1,** *270 (1943)*; **7,** *275 (1949)*; "Recommended Dietary Allowances", *National Academy of Sciences-National Research Council, publication 302, (1953)*).

Unfortunately, no data could be made available on the prevalence of infantile beriberi in children of mothers in this test, but the authors stated that it was not as high as would be expected from the low thiamine values. They point out that these children also have a low caloric intake which may make their thiamine requirements considerably less. I. Concepcion and R. L. Dee (*Philippine J. Sci.* **78,** *373 (1949)*) reported that the thiamine content of milk from mothers whose children showed signs of infantile beriberi was especially low, even after several months of lactation (an average of 4.97 micrograms per 100 ml.).

The thiamine content of human milk in Malaya, and the effect of the administra-

tion of supplementary thiamine, was studied by I. A. Simpson and A. Y. Chow (*J. Trop. Pediat.* **2,** *69 (1956)*; **3,** *110 (1956)*). They used over 100 women of three racial groups (Malayan, Chinese, and Indian) in their over-all studies. Several of these mothers, or their infants, showed clinical indications of beriberi. From studies with 26 women of this group the authors concluded that, although either orally or parenterally administered thiamine usually resulted in increased milk levels, there were marked variations in responses of different women. The highest thiamine level obtained was 38.9 micrograms per 100 ml. following daily injection of 40 mg. of thiamine for six days. Without supplementation of thiamine some milks were as low as 2.3 micrograms even after several months of lactation. In a few instances no thiamine could be found in some of the samples obtained during the early stages of lactation.

In conclusion, the evidence is clear that in certain areas of the world, especially in the rice eating areas, the thiamine intake of many mothers and infants is extremely low. Additional studies are needed on the relationship of thiamine intake to incidence of beriberi in infants as evidenced by biochemical and clinical tests, but it is clear that infant beriberi does exist in certain areas of the world.

AMINO ACID SUPPLEMENTATION OF RICE

Amino acid supplementation of rice has been studied quite extensively with growing rats as the experimental subjects, and both beneficial and adverse effects of amino acid supplements have been demonstrated (*Nutrition Reviews* **14,** *225 (1956)*). Perhaps the most striking observation was that neither lysine nor threonine alone proved beneficial as supplements to rice, whereas the combined addition of these two amino acids produced a marked growth response. The other feature of some interest was that a number of combinations of amino acids actually depressed the growth rate. In order to determine whether similar results might be obtained with man as the experimental animal, J. M. Hundley, H. R. Sandstead, A. G. Sampson and G. D. Whedon (*Am. J. Clin. Nutrition* **5,** *316 (1957)*) performed a series of experiments on four young adult volunteers fed diets in which rice was the major source of protein.

The basal diet consisted of rice, 250 or 350 g. and fat (either butter or margarine) 100 g. with fruit and sugar added so the diet provided 4.31 to 5.07 g. of nitrogen and 2,800 to 3,500 calories daily. Each subject also received a vitamin capsule daily. From 65 to 83 per cent of the nitrogen came from the rice proteins, the only animal protein being that contained in the butter or margarine. The subjects were allowed 18 to 30 days to adjust to the diet, when an amino acid mixture was added for 12 or more days. A nitrogen balance technique was used to determine the effects of the amino acid supplements. The experiments were continued for 15 to 19 six-day metabolic periods, with periods on the amino acid supplements being alternated with periods on the basal diet. Urinary excretion of arginine, lysine and threonine was determined, in four series of experiments, on the last two days of each six-day metabolic period. The levels of these three amino acids in serum and in serum proteins were also determined. A number of other clinical determinations were made throughout the test periods.

The amino acid supplements tested included L-threonine, 0.62 g. daily; L-lysine, 0.62 g. daily; a combination of L-threonine, 0.38 g., and L-lysine, 0.62 g.; this combination with either 1.0 g. of DL-methionine or 0.6 g. of L-histidine hydrochloride; two mixtures of the eight essential amino acids to

provide 0.82 or 0.92 g. of nitrogen daily (one containing lower levels of lysine and threonine than the other); a mixture of non-essential amino acids to provide 0.82 g. of nitrogen and a second mixture to provide about the same amount of nitrogen as the individual supplements of lysine and threonine.

When the subjects were first given the rice-fruit diets they showed markedly negative nitrogen balances with a gradual trend toward nitrogen equilibrium as the experiment was continued (*Nutrition Reviews* **9,** *73 (1951)*; **10,** *46 (1952)*). Positive balance was achieved in only a few instances even after supplementation with amino acids. This made it necessary to evaluate the effect of the amino acid supplements against previous and subsequent periods on the basal diet and against the general trend toward nitrogen equilibrium. This was carefully done by statistical methods in order to determine the significance of the responses to the various supplements.

The results with the threonine-lysine mixture were not uniform from individual to individual; nitrogen balance was significantly improved in one subject, somewhat improved in a second, unchanged in two others, and in the fifth there was a highly significant negative response. In one trial out of three an improvement was observed upon adding methionine with threonine and lysine, and in one trial the response to threonine, lysine and histidine was better than the response to only lysine and threonine. Lysine and threonine were tested singly in only one subject in whom a positive response was obtained with lysine and none with threonine.

With the essential amino acid mixtures the results were not appreciably different from those obtained with the mixture of lysine and threonine. In one trial a mixture of essential amino acids equivalent to Rose's "minimum" requirements produced a positive shift in nitrogen balance but did not induce a positive balance. In another case a slight improvement was noted, but in a third a highly significant negative response occurred. A non-essential amino acid mixture, equal in nitrogen content to the essential amino acid mixture, was as effective as the essential amino acid mixture but a mixture of non-essential amino acids isonitrogenous with the lysine or threonine supplements was without effect.

The subjects' sense of well-being was unaffected by the amino acid supplements. During the course of the experiment two of them showed a decrease of 1 g. in hemoglobin and one showed a decrease of 1.5 g. in serum proteins. There were no appreciable blood changes in the others. There was no change in the arginine, lysine or threonine content of the whole serum or of the serum proteins in the three subjects studied.

It is evident, as the authors point out, that the amino acid mixtures were much less effective than would be expected from the results of animal experiments. They noted, from a comparison of the minimum requirements with the amounts of amino acids supplied in the rice, that lysine and methionine were the most likely amino acids to become limiting and that these two produced some positive response. However, in view of their results with the non-essential amino acids, they concluded that nitrogen, as such, was the main limiting factor. Since young growing rats were used in the animal experiments, which were the basis for this study, it should not be concluded that the lysine-threonine mixture would prove ineffective in the young growing child consuming mainly rice. Only with similar experiments on younger subjects can this point be decided.

The observation that in relatively few cases was positive nitrogen balance obtained may not be as significant as would appear at first sight. The subjects had been consuming about 85 g. or more of protein per day prior to the experiment and in most cases were only just approaching balance with the lower protein intake toward the end of the experimental period of about 100 days.

Thus the positive responses would probably be of much more significance over a longer period of time, and it is of interest that responses to amino acid supplements in subjects on low-protein, vegetable diets have been more clearly shown after severe depletion (*Ibid.* **14,** *291* (*1956*)).

Finally it should be noted, as the authors emphasized, that there was not a "uniform pattern of response" to the amino acid supplements used. In a number of instances no response was obtained, and in at least two cases significant adverse effects were observed when beneficial effects might have been expected. No methodical study of the occurrence of amino acid imbalance in man has been made and whether these two examples represent imbalances of the types observed in experiments on animals (*Ibid.* **14,** *225* (*1956*)) is not clear. Nevertheless, the fact that these two cases were observed emphasizes the need for extensive studies of the effects of amino acid supplements in the diets of man, particularly in low protein diets consumed by growing children.

HEREDITARY GLYCINURIA

With the development of paper chromatography, workers the world over are studying amino acid patterns in the urine, and it may be expected that new entities will be described from time to time. Two recent reviews have summarized the information on urinary amino acid excretion in infants and children (*Nutrition Reviews, Jan. 1958,* "Urinary Amino Acid Excretion in Infants") and a variety of aminoacidurias in man (*See* p. 39 of this issue, "Urinary Amino Acid Excretion in Disease"). In addition to the generally recognized syndromes discussed in the latter, a bewildering array of other aminoacidurias has been described from time to time, usually recognized in only one or a few patients, and often incompletely studied. References to these will be found in the papers covered in the above reviews.

A. de Vries *et al.* (*Am. J. Med.* **23,** *408* (*1957*)) report careful studies on an apparently new entity under the title "Glycinuria, a Hereditary Disorder Associated with Nephrolithiasis." Their patient, a 20-year-old Bulgarian-born female resident of Israel, had had episodes of pus in the urine beginning at the age of two, kidney colic beginning at four, and a kidney stone at six years. Several stones were removed at ten, and the left kidney was removed at age 11. She was well until the age of 19, when hematuria and pain on the right side led to discovery of a renal calculus, eventually removed at age 21 because of progressive enlargement and development of hydronephrosis. Paper chromatograms of the urine showed a prominent glycine spot without other abnormality. The 24-hour excretion of glycine measured by microbiological assay on six consecutive days was 798 to 1200 mg. (average 991) compared to normal values of 113 to 218 mg. (average 156) by this method. The daily total amino nitrogen excretion was 241 to 316 (average 286) compared to the normal of 51 to 152 mg. (average 120); the increase was accounted for by the excess glycine. Since the serum amino acid pattern was normal, with no increase of the glycine spot on chromatograms, the authors classified their case with the renal aminoacidurias. They therefore studied the urinary excretion of an intravenous glycine load (7 g. in 70 ml. saline in 2½ minutes). The patient excreted 850 mg. glycine in 20 minutes and 1,240 mg. in 200 minutes, compared with two normal subjects who excreted 240 and 285 mg. in 20 minutes and 394 and 340 mg., respectively, in 200 minutes after a similar glycine load. A chromatogram of the patient's serum showed a return of glycine concentration to normal in about two hours. A low-protein diet (30 g. per day) did not depress glycine excretion on the third day.

The renal calculus consisted chiefly of calcium oxalate. Its glycine content was estimated by chromatography of a hydrochloric acid extract of the calculus as about 0.5 per cent of the dry weight of the stone. No glycine was detectable by the same method in oxalate stones from other urologic patients, nor did an oxalate calculus soaked in patient's urine containing 1 part per thousand of glycine show any glycine when subjected to the same analysis.

The authors could find no evidence to suggest conversion of glycine to oxalate. Urinary calcium excretion was normal and an intravenous calcium infusion test gave results consistent with normal parathyroid function. The phosphate/creatinine clearance ratio was normal. No glucosuria was demonstrable even after oral glucose. Xanthine excretion was normal.

Members of the patient's family were studied and excessive urinary glycine excretion with otherwise normal amino acid patterns were demonstrated in the sister, mother, and maternal grandmother. The father, maternal uncle, and two-year-old daughter of the sister had normal urinary glycine excretion. The sister, aged 22 years, had a renal calculus. There was a history of renal colic in the grandmother with glycinuria, but a calculus was not demonstrable. From the limited evidence the authors infer that the glycinuria is probably a dominant sex-linked genetic trait.

De Vries and his colleagues calculated their patient's renal glycine clearance to be 71 per cent of the creatinine clearance, compared to a normal ratio of about 7 per cent. In other words, 71 per cent of the glycine in the glomerular filtrate (or ten times the normal) was excreted in the urine. The authors point out that the syndrome they describe is essentially different from the De Toni-Debré-Fanconi syndrome, hepatolenticular degeneration (Wilson's disease), the so-called "H syndrome" and the reported cases of osteomalacia with predominant glycinuria. The lack of excretion of other amino acids, glucosuria, or phosphaturia distinguishes the syndrome they describe from other known entities and points up deficiencies in various existing hypotheses of glycine excretion. Evidently the tubular reabsorption of glycine is a very specific one, contrasting with the loss of four acids in cystinuria and the associated loss of many amino acids, phosphate, and glucose in Fanconi's syndrome.

The relation between glycinuria and the formation of oxalate calculi appears more than fortuitous, yet an explanation is not at hand. The urine was far from saturated with this acid. Glycine could be extracted from the stone by normal hydrochloric acid but not by water, yet the acid extraction was considered too mild to release peptide or protein glycine. Cystinuria is not an analogous condition; here cystine calculi form in urine supersaturated with this amino acid.

The authors do not state why they studied the amino acid excretion of their patient; one infers that it was fortuitous. Much survey work remains to be done by paper chromatography of plasma and urine amino acids in various states. As new disease entities are added to the list of aminoacidurias of man, these may well throw more light on renal tubular function than on amino acid metabolism, for many aminoacidurias are evidently abnormalities of tubular reabsorption (*e.g.*, Fanconi's syndrome) and some (*e.g.*, cystinuria) are also genetically determined. The inheritance of specific enzyme systems is presumably at fault.

The evidence appears conclusive that cystinuria is a defect of tubular reabsorption of the amino acids cystine, lysine, ornithine, and arginine, with formation of concretions of the least soluble one, cystine (which is not the most abundant). Infusion of a lysine load in the normal human subject results in gross increases in urinary excretion of all four acids (*Nutrition Reviews* **15,** *267 (1957)*). P. D. Doolan, H. A.

Harper, M. E. Hutchin and E. L. Alpen *Am. J. Med.* **23,** *416 (1957)*) have reported (on the renal clearance of lysine in cystinuria. Under lysine load, its clearance approaches that of inulin.

In summary, a new entity has been described, glycinuria associated with formation of oxalate renal calculi, apparently inherited as a sex-linked dominant trait. Plasma glycine levels were normal and the glycinuria is evidently due to impaired renal tubular reabsorption of glycine. There is no glucosuria or phosphaturia, and the reason for the stone formation is not known.

LYSINE AND FEATHER PIGMENTATION

Deficiencies of individual amino acids in the diet are not usually characterized very obviously according to the particular amino-acid that is deficient. In contrast to this is the characteristic appearance of a white barring across the wing feathers of young turkeys reared on a diet deficient in lysine. Nothing similar is seen when turkeys are reared on diets deficient in any of the other amino acids. In view of this, it seems a possibility that the lysine molecule is required at a high level for feather pigmentation, not only for the usual synthesis of proteins but also for metabolism in another direction. If this were the case it might also follow that some molecules similar to lysine, but of no use for protein synthesis, might still be active in the prevention of "white barring", and so yield a clue as to this conjectural second role of lysine.

P. Vohra and F. H. Kratzer (*Arch. Biochem. Biophys.* **68,** *76 (1957)*) have now investigated this possibility by feeding a series of seven compounds, all similar in some respects, to lysine, to lysine-deficient poults. From three days of age to 23 days the poults that received the control diet without supplement showed an average daily gain of approximately 2.3 per cent of their body weight and almost all the flight feathers that developed were barred. Birds that received the same ration supplemented with 6 millimoles of DL-lysine hydrochloride per 100 g. of ration showed a daily gain of approximately 6 per cent, and one-third of the incidence of barring seen in the control birds. Supplementation of the control diet with either L-proline, L-ornithine, DL- homocitrulline or ϵ-aminocaproic acid in equivalent quantities had no effect on either the rate of growth or the incidence of white bars in the flight feathers. The addition of DL-α-aminohexanoic acid seemed to depress growth, and very little feathering of any kind was obtained. A mixture of 4.5 millimoles of DL-lysine and 1.5 millimoles of DL-pipecolic acid was no more active in either respect than the 4.5 millimole level of lysine given by itself. Lastly, the addition of various levels of ϵ-N-acetyl-DL-lysine showed approximately 30 per cent of the growth stimulus that was obtained with equivalent quantities of DL-lysine, and also a slight reduction in barring when it was fed at the level of 12.2 millimoles per 100 g. of ration.

The authors conclude therefore, that, in its effect on feather pigmentation, lysine cannot be acting as a substrate for melanin production with pipecolic acid as an intermediate metabolite, a conversion that has been observed in *Neurospora* as well as animal tissues. Nor do the specificity requirements for feather pigmentation seem any less rigid than those for growth. Removal of either the α-NH_2 or the ϵ-NH_2 group from the lysine molecule makes it inactive for either purpose. A blocking of the ϵ-NH_2 group as in the homocitrulline molecule also makes it quite inactive. Even acetylation of the ϵ-NH_2 group greatly reduces the activity for poults. This is interesting because A. Neuberger and F. Sanger (*Biochem. J.* **37,** *515 (1943)*) found ϵ-N-acetyl-lysine to be as

active as lysine in growth assays with rats. On the other hand the present finding seems to be in line with the generally agreed hypothesis that much of the nutritional damage occurring when protein foods are heat-processed is due to the formation of indigestible links between the ϵ-NH_2 groups of the lysine in the protein and either free carboxylic acid groups in the protein or active groups in any carbohydrates present, as illustrated in the work of N. R. Eldred and G. Rodney (*J. Biol. Chem.* **162,** *261 (1946)*) and J. Mauron (*Rev. Int. Vitaminologie* **27,** *85 (1957)*).

MEASUREMENT OF ENERGY CONTENT OF FOODSTUFFS

A simple and reproducible biological method for determining the energy content of foods has been described by E. E. Rice, W. D. Warner, P. E. Mone and C. E. Poling (*J. Nutrition* **61,** *253 (1957)*) which may, under many conditions, obviate the need for more complex and time consuming physical and chemical methods (*Nutrition Reviews* **14,** *306 (1956)*).

The basic concept underlying the method, is that animals consuming adequate amounts of all essential dietary constituents, except calories, should gain weight in proportion to the number of calories ingested. In essence, the method consists of measuring the growth response of young rats, reared under conditions of energy restriction, to increments of a food.

The original basal diet devised by the authors had the following percentage composition: casein, 43.82; sucrose, 13.14; celluflour, 3.29; salt mixture, 6.57; cornstarch, 30.44; vitamin E supplement, 1.42; and vitamin mixture, 1.32. The rats were given, in addition, a weekly supplement of the fat soluble vitamins. During the investigation, in which a number of types of fats were tested, it was found that even in short assay periods there appeared to be some response to essential fatty acids. Therefore, in order to avoid errors that might arise from this source, four parts of destearinized cottonseed oil were mixed with 96 parts of the basal diet. Weanling rats fed 5 g. of the basal diet per day gained between 5 and 10 g. per week. The provision of adequate calories with this amount of the basal diet permitted gains of over 100 g. in three weeks.

The experimental animals were male white rats. They were reared for seven days in individual cages, during which time they were allowed only 5 g. of the basal diet per day. At the end of this period they were distributed into uniform groups of four rats each. One group continued to receive 5 g. of the basal diet. The others were given, in addition to the 5 g. of basal diet, carefully weighed quantities of various foodstuffs. Initially the rats were weighed three, seven, 14 and 21 days after being fed the test diets. When it was found that the responses were uniform and that the weights at seven days were as reliable as those obtained in longer experiments, subsequent tests were limited to seven days.

In the initial study the responses to graded levels of lard and to two levels of sucrose were determined. Each series was replicated three times with both seven and 21-day weights being reported. Weight gain was a function of the number of calories provided. A plot of the results with different levels of lard gave a smooth curve with gradually decreasing increments of weight gain for each increase in the amount of lard fed. This would be expected because the maintenance requirement for calories would increase with increasing rate of gain. Equivalent numbers of calories provided as sucrose or lard gave equivalent weight gains.

Subsequently the growth responses to sucrose, glucose, casein, celluflour and individual fatty acids were determined and the

calorie equivalents were calculated from a reference curve prepared for each experiment from the weight gains obtained with various levels of lard. The metabolically available energy from the lard was taken as 9.0 calories per gram calculated from a heat of combustion of 9.5 calories per gram and a digestibility of 95 per cent. For all of the highly digestible components the calorie-equivalents determined experimentally agreed well with the theoretical calorie contents. Fecal analyses indicated that the group of fatty acids varied in digestibility from 11 per cent for stearic acid to 88 per cent for oleic acid. The availability of calories as estimated from the reference curve agreed well with the digestibility values.

Also of interest is the observation made by the authors that a temperature differential of 4 degrees, between the top row of cages and the bottom row of cages on their racks, resulted in a measurable difference in the rate of gain, per gram of food consumed, of groups of rats reared in the different rows in the racks. This observation indicates the care that must be taken in conducting experiments to determine calorie utilization.

This technique provides a rapid and simple method of estimating the available energy content of foods and obviates the need for determining digestibility, body storage, and the excretion of endogenous materials. The authors make no claim that it will serve to replace the more exacting studies required when the metabolic relationships of various dietary constituents are being studied, but they do point out that it will prove valuable "in many types of research, where it is necessary to detect changes in nutritive value relating to fat, protein or carbohydrate".

PASSAGE OF IODINE THROUGH THE PLACENTA

It has been demonstrated in several species that, during certain late stages of development, the thyroid of the fetus will fix iodine injected into the maternal organism (A. Gorbman *et al.*, *Endocrinology* **51,** *546 1952*)). The reverse transfer from the fetus to the mother will also take place, but this occurs to a lesser extent as the gestation age increases (P. F. Hall and N. B. Myant, *J. Physiol. London* **133,** *181 (1956)*). It has been demonstrated further that L-thyroxine and L-3:5:3′ triiodothyronine circulating in the blood of the mother will traverse the placenta (M. M. Grumbach and S. C. Werner, *J. Clin. Endocrinol. and Metab.* **16,** *1392 (1956)*).

An attempt to investigate more closely the role of the placenta in these phenomena was recently made by B. Nataf, M. Stez, R. Michel and J. Roche (*Bull. Soc. Chim. Biol.* **39,** *233 (1957)*). Radioiodine (NaI), with and without carrier and L-3:5:3′ triiodothyronine were injected into rats toward the end of gestation (fetus size: 3 to 4 centimeters), and also into fetuses only. The animals were sacrificed at different intervals and various organs were examined, both of the fetus and of the mother, for content of I^{131}. The passage of $Na^{24}Cl$ through the placenta was also measured in some instances.

When I^{131} was injected into the muscle of the mother it was found that the radioactivity of the fetal plasma became greater than that of the maternal plasma in less than one hour. By ten hours the activity in the fetal plasma was two and one half times greater than that in the maternal plasma. The radioactivity of the placenta was also greater than that of the maternal plasma, and had about the same activity as that of the fetal plasma. When the amount of injected NaI was increased to 1 mg. by adding carrier, the activity of the fetal plasma and the placenta relative to the maternal plasma was reduced by more than one half. In this

respect the mechanism of the iodine concentration in the placenta is similar to that of thyroid fixation of the same ions.

Intravenous injection of L-3:5:3′-triiodothyronine resulted in slower movement of I^{131} into the fetal plasma from the maternal plasma than was the case when I^{131} was injected alone. The concentration of isotope in the placenta was even less than in the fetal plasma.

L-3:5:3′-triiodothyronine injected intramuscularly into the mother in a physiological dose (0.2 mg.) was also found to pass into, and through, the placenta. When this substance was injected into the muscles of the fetus an even lower concentration appeared in the maternal plasma on a unit volume basis, whereas the concentration in the placenta was approximately equal to that in the fetal plasma. The authors fail to point out, however, that the total radioactivity of the maternal plasma probably would exceed that of the total fetal plasma. This would indicate considerable passage of L-3:5:3′-triiodothyronine in the reverse direction through the placenta.

When I^{131} without carrier, I^{131} with carrier (1 mg NaI), and L-3:5:3′ triiodothyronine were separately injected into different groups of pregnant rats, it was found that the inorganic iodine failed to concentrate in the uterus, ovaries, kidneys or adrenals. However, the L-3:5:3′ triiodothyronine was found to be concentrated in all these maternal tissues, with the exception of the uterus.

The authors are of the opinion that the placenta acts to concentrate iodine for the fetal organism, thus serving in some manner as a "pump" for this particular element. Certainly a high concentration of iodine occurred in the placenta and fetal serum relative to the maternal plasma, although this was not the case for sodium.

It seems possible that the placenta has a special affinity for ionic iodine but is nevertheless periodically in equilibrium with both maternal and fetal blood. This could lead to the eventual appearance of relatively more iodine in the fetal plasma than in the maternal plasma because of the difference in dilution factor. It is also possible that embryonic tissue has a special affinity for ionic iodide and that the resulting high concentration of iodine in the fetal plasma (and perhaps even that of the placenta) may be due to the equilibration of this tissue with the adjacent plasma. A third possibility is that the placenta acts to combine the ionic iodine with some other molecule on the fetal side of the circulation. This complex might not readily traverse the placental barriers and hence would appear chiefly in the fetal circulation. With this latter possibility in mind, it would seem to be desirable (1) to analyze maternal placenta as distinct from fetal placenta and (2) to determine the chemical forms in which the fetal iodine is circulating.

FOOD INTAKE AND WATER RELATIONSHIPS

It has been postulated that hydration of adipose tissue is associated with hunger, and dehydration with satiety (Hoelzel, *Am. J. Digest. Dis.* **12,** *156 (1954)*). In order to investigate the relationship between food intake and water consumption S. Lepkovsky and co-workers (*Am. J. Physiol.* **188,** *327 (1957)*) measured food intake, stomach emptying time, water content of a variety of body structures, and water content of gastric and intestinal contents of rats allowed to eat either with or without access to water.

The experimental procedure consisted of training a number of rats weighing between 250 and 350 g. to eat their total daily food supply within two hours. Then on the day of the experiment the rats were divided into

two groups, one of which was allowed free access to water while the other was deprived of water. Both groups were offered a diet containing ground whole wheat, 62; whole milk powder, 10; casein, 15; soya bean oil, 10; calcium carbonate, 2; and sodium chloride, 1. Groups of animals were killed immediately after they had eaten and at two, five and nine hours after the meal. The stomach and intestinal contents were removed and samples of several organs were taken for the determination of moisture and dry weight.

The group of rats deprived of water ate about 10 g. of diet, whereas those allowed free access to water ate about 14 g. and consumed approximately 14 ml. of water (*Nutrition Reviews* **13,** *157 (1955)*). Remaining very close to 50 per cent over the entire experimental period of nine hours, the percentage of water in the stomach contents was amazingly constant regardless of whether the animals had access to water. Also, the rate of stomach emptying, expressed as a percentage of the food eaten, remained the same regardless of the accessibility of water to the animals. About 40 per cent of the ingested food had disappeared from the stomach within two hours, 60 per cent had disappeared within five hours, and about 20 per cent remained at nine hours. Very little variation was noted in the dry weight of the intestinal contents regardless of the amount of food eaten or whether the animals had access to water. Thus, it was evident that gastric emptying was controlled in such a way that at no time did the intestine contain an excessive quantity of solids. It would appear from this that digestion and absorption were exceedingly rapid during the first two to four hours after food was ingested and that the rate fell off gradually with time.

Analyses of the various organs and body structures were performed in order to determine whether the moisture content of the gastrointestinal contents was maintained at the expense of any particular body component. In the groups that were allowed to drink freely there was a decrease only in the moisture content of the skin and the gastrointestinal tract. In groups given no water, the moisture content of both skin and muscle fell, the former by about 6 per cent, the latter by 2 per cent; also the moisture content of the stomach dropped from 77 to 72 per cent and that of the intestine from about 74 to 72 per cent. The other organs and structures (liver, bone, kidney, heart, blood and brain) showed relatively little change. The moisture values were obtained on pooled samples and no estimate of the range of values from the different experiments was presented. However, the water content of the adipose fat was measured on several samples at each time interval and individual values were given. The variation among the individual values was very large but the average of all values indicated that there was a loss of water from these fatty tissues after eating.

Since the percentage of moisture in the stomach contents was the same whether or not the rats were allowed access to water, and since the food intake of rats receiving no water was substantially less (roughly 30 per cent less) the results are in agreement with the idea that food intake may be regulated by changes in the moisture content of the tissues. Ingestion of food resulted in dehydration, particularly of skin (Cori., *J. Biol. Chem.* **66,** *691 (1925)*), gastrointestinal tract, and possibly muscle and deposits of adipose fat. These shifts in moisture content were most clearly evident in the groups that received no drinking water but the same trends were apparent in results for groups allowed to drink freely. However, it is difficult to see, as the authors point out, how these tissue changes can stimulate the "feeding" and "satiety" centers of the hypothalamus (*Nutrition Reviews* **9,** *157 (1951)*) in view of the essentially constant moisture content of the blood. The possi-

bility that changes may have occurred in blood glucose level was not examined (*Ibid.* **11,** *55 (1953)*); **13,** *299 (1955)*).

Estimations were made only at the end of the two hour feeding period (zero time) and at two, five and nine hours later. It is therefore possible that important changes may occur during the feeding period which would not be observed using this technique. A much shorter feeding period, with more frequent observations during the period immediately after food ingestion, would provide information on this point and would show whether some of the variations observed in the values for brain are of more significance than would presently appear to be the case. It is possible that transient changes in the moisture content of the brain could affect food consumption.

Some emphasis is placed by the authors on the constancy of the moisture content of the gastric contents, regardless of the amount of water or food consumed by the rats. However, no consideration is taken of the fact that only a single diet was fed throughout. It would seem plausible that osmotic relationships might play an important part in determining the amount of water drawn into the stomach after the ingestion of food and that the moisture content would vary considerably depending upon the osmotic pressure exerted by the diet constituents.

Further studies of this nature, using a variety of conditions, will expand our knowledge of factors influencing food intake, digestion, absorption and water relationships within the body. It is to be hoped that this study represents the initial results of a long-term project.

METABOLISM OF ESSENTIAL FATTY ACIDS

Linoleic, linolenic and arachidonic acids are the three most widely recognized members of the group of higher polyunsaturated fatty acids exhibiting essential fatty acid activity. However, the biological activity of linolenic is known to differ considerably from that of linoleic and arachidonic acids (*Nutrition Reviews* **10,** *154 (1952)*), which suggests that the metabolic fate of linolenic acid is different from that of the other two fatty acids. On the assumption that information about the metabolism of linoleic acid is necessary in order to explain these differences in biological activity, G. Steinberg, W. H. Slaton, Jr., D. R. Howton and J. F. Mead (*J. Biol. Chem.* **224,** *841 (1957)*) have studied the fate of isotopically labeled linoleic acid in the animal body.

About 90 mg. of a mixture containing 40 per cent of methyl linoleate-1-C^{14} and 60 per cent of corn oil were administered to each of a group of ten rats. Eight of the ten were killed after four hours. The other two were retained for respiratory carbon dioxide estimations. The livers, kidneys, hearts, spleens and abdominal fat of the animals were pooled and the higher unsaturated fatty acids were isolated as insoluble polybromides. These were debrominated, hydrogenated and separated by reversed phase chromatography. The arachidic acid (C_{20}) obtained was degraded in such a way that the radioactivity of each of the four carbon atoms, beginning with the carboxyl carbon, could be determined separately. The radioactivity of the carboxy-carbon of the stearic acid was also determined. The linoleic acid was isolated and purified and a fraction of the higher polyene mixture was isomerized.

As much as 30 per cent of the carboxy carbon of linolenic acid was recovered in the respiratory CO_2. However, the low value of 6 per cent for one rat indicates the variability that may be encountered. The measurement of radioactivity in the various lipid fractions isolated indicated that the

carboxyl carbon from linolenic acid had been incorporated into the fatty acids of the saturated fraction and those of the various unsaturated fractions.

In sequence of steps, degradation of the C_{20} acids isolated from the unsaturated fatty acid fraction yielded the following information: the carboxyl carbon contained 27 per cent of the total radioactivity present in the C_{20} fraction and, counting from the carboxyl end, C_2 contained 8 per cent; C_3, 60 per cent; C_4, 4 per cent and the 16 carbon residue, 1 per cent. A comparison of the distribution of the labeling in the C_{18} acids isolated from the unsaturated fatty acid fraction with that in the original linolenate revealed that there was labeling beyond the original label in the carboxyl carbon, thus indicating that the labeling in C_4 and in the C_5 to C_{20} chain was real. A similar distribution pattern was found in the carbon skeleton of the polyene mixture. It appeared from these results that the linolenate had undergone several β-oxidations without affecting the double bond system and that the molecule was rebuilt from acetate, some of which arose from β-oxidation of the labeled linolenate. In similar experiments in which carboxy-labeled linoleate was used no radioactivity was detected beyond carbon atom 3 of the C_{20} acids (Steinberg, Slaton, Howton and Mead, *Ibid.* **220,** *257 (1956)*).

When the ultraviolet absorption characteristics of pure methylarachidonate were compared with those of the isomerized C_{20} polyunsaturated fatty acid fractions from linoleate-fed and linolenate-fed animals, it was evident that there was an excess of from 3.5 to 6.0 per cent pentaene or hexaene acids in the fat from the linolenate-fed animals. These observations conform with the results of R. Reiser (*J. Nutrition* **42,** *325 (1950)*). Some presumptive evidence from columns in which incomplete separation of a portion of the C_{20} unsaturated fatty acids was obtained, indicated that most of the radioactivity in this fraction was present in the C_{20} pentaene acids. There was very little activity in the linoleate isolated directly from the main bromination mixture.

In summing up their observations, the authors state that if linolenate is converted to arachidonate it is not reduced to linoleate as the first step. Thus, if the Δ^{15} double bond is reduced, it evidently is not reduced until after the chain lengthening step has occurred. In view of the evidence from this study and that of others regarding the conversion of linolenate to more highly unsaturated fatty acids (Reiser, *loc cit.*; Houton *et al.*, *J. Am. Chem. Soc.* **76,** *4970 (1954)*) it seems likely that linolenate "is converted to C_{20} and C_{22} pentaene and hexaene acids, possibly via a C_{20} tetraenoic acid isomeric with arachidonic acid". Thus the difference in the biological activity of linoleate and linolenate (Hume *et al.*, *Biochem. J.* **34,** *879 (1940)*; Greenberg *et al.*, *J. Nutrition* **41,** *473 (1950)*; *Thomasson. International Conference on Biochemical Problems of Lipids, Brussels, p. 212 (1953)*) would appear to have a metabolic basis.

NUTRITIVE VALUE OF PROTEINS

One widely used method of measuring the nutritive value of protein is that proposed and developed by T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **37,** *223, 557 (1919)*; **41,** *275 (1920)*). This method of protein evaluation is relatively simple, is expressed as the gain in body weight per gram of protein consumed, and is defined as the protein efficiency ratio. However, this method has received critical attention (H. H. Mitchell, *Ind. Eng. Chem. (Anal. Ed. & News Report* **16,** *696 (1944)*) because, in the hands of many investigators, no systematic attempt was made to find the dietary

level of a given protein that would yield maximum efficiency (maximum gain per gram of protein consumed).

In some recently reported studies at the University of Arkansas, Fayetteville, an attempt has been made to evaluate factors that may influence the protein efficiency ratio (B. Sure, *J. Agr. Food Chem.* **3,** *789 (1955)*). Studies were conducted on a number of cereal grains (wheat, rye, yellow corn, grain sorghum, barley and rolled oats), oil meals (peanut, soybean, and cottonseed), dried nonfat milk solids, and dried whole egg. These foods were fed in a ration in which the protein source was varied between 5 and 12 per cent. The studies were also varied in length between four and ten weeks. Wistar strain rats, 28 to 30 days of age, served as experimental animals.

The ration, in addition to the 5 to 12 per cent of protein, contained the following, in per cent: salt mixture, 4; hydrogenated vegetable shortening, 7; cod liver oil, 2; wheat germ oil, 1; and glucose (cerelose) to equal 100. All known B-vitamins required by the rat were included as a supplement.

Although the result is not surprising, a decreasing protein efficiency ratio was demonstrated with prolongation of the experimental period. For example, with dried whole eggs the four-week experimental period yielded a protein efficiency ratio of 3.4, while an eight-week test yielded a value of 2.7. Dried nonfat milk solids declined from an efficiency ratio of 2.7 in a four-week test to 1.9 in a ten-week test. It is of interest that the plant protein sources were influenced less with regard to their efficiencies by lengthening the experimental period. Soybean meal and cottonseed meal, for example, had protein efficiency ratios of 1.5 in a four-week test and decreased to only 1.3 in 10-week tests.

From these results it is apparent that protein efficiency ratios can vary, depending upon the feeding period, and this may, in part, account for the variable results that can be found in the literature.

Although the results were not entirely uniform, in general the protein efficiency ratios decreased with increasing concentration of protein in the ration. With egg protein, for example, a ratio of 2.6 was found at the 5 per cent protein level in the ration, compared with a ratio of 1.8 at the 12 per cent protein level. Dried nonfat milk protein declined from a ratio of 2.3 at the 5 per cent protein level to 1.6 at the 12 per cent protein level. Rolled oats yielded essentially the same protein efficiency ratio (1.6–1.7) at 7, 8, 10 and 12 per cent dietary protein levels. Wheat tended to improve with increasing protein level in the diet (ratios of 0.8 and 1.1 for 7 and 9 per cent protein, respectively).

As a continuation of these studies, a more recent report evaluates the influence of dietary protein levels ranging from 15 to 30 per cent (Sure, *Ibid.* **5,** *463 (1957)*). The foods tested for their protein efficiency ratio, of course, had to be higher in protein than the cereal grains. The foods selected were corn gluten meal, cottonseed meal (after removal of gossypol), defatted soybean flour, wheat germ meal, defatted wheat germ, dried nonfat milk solids and defatted whole eggs. In all foods tested the protein efficiency ratio was at the maximum when the protein in the diet was at the 15 per cent level, and the ratios were essentially the same or only slightly less than those obtained in the earlier study when the protein was present at a dietary level of 12 per cent (cottonseed, soybean, milk and egg protein.)

These studies are of interest in that they illustrate that protein efficiencies vary, depending on their dietary level, and this may have practical application in livestock feeding or in human nutrition. For example, at the 15 per cent level of intake, the protein efficiency ratios of defatted soybean flour (1.2) and cottonseed meal

(1.2) were far superior to that of corn gluten meal (0.8), while at the 25 per cent level of intake the efficiency of corn gluten protein (0.7) was appreciably higher than those for the oil meals (0.5–0.6).

These studies merit attention since too often protein efficiency ratios reported in the literature are assumed to represent maximum values whereas the conditions under which they were determined, such as length of experimental period and dietary protein level, have a significant influence.

FEEDING PATTERNS IN MICE

An animal may be trained to operate a device or perform a function if it is rewarded as soon as it behaves in the specific manner. This principle, known as operant conditioning, has been described by B. F. Skinner (*Proc. and Papers of 13th International Congress of Psych. Stockholm (1951)*). J. Anliker and J. Mayer (*J. Applied Physiol.* **8,** *667 (1956)*) have adapted operant conditioning to the study of feeding patterns of mice. Use of this procedure enabled them to study short term variations in feeding habits of mice, while avoiding the impracticability of frequent measurements of food intake.

Mice were made obese by the production of lesions in the ventromedial nuclei of the hypothalamus, either directly by surgery or indirectly by the administration of goldthioglucose. Hereditary obesity was studied in Obob mice and all three types of obese mice were compared with normal controls. Anliker and Mayer observed that a mouse housed in a cage equipped with a suitably placed sensitive lever would operate the lever at a relatively constant rate in spite of the absence of a food reward. This response rate was known as the free operant level. By rewarding lever presses with food the animal became conditioned to responding with more frequent lever pressing activity until his hunger was satisfied. A period of minimal response ensued, followed by the regaining of appetite and the eventual resumption of lever pressing activity. By recording the number of pellets delivered, and the time of delivery, it was possible to chart feeding activity and to observe the duration and characteristics of a complete feeding and fasting cycle.

The apparatus employed consisted of a box divided into two compartments, one being the cage and the other containing the automatic feeder. The lever, located on the partition was adjusted as to shape and leverage so that 4 g. was sufficient force to operate the switch. Care was taken to have the lever travel just far enough to operate the switch since over-travel would reduce the maximum rate of lever pressing which could be attained. On the other hand the sensitivity was necessarily limited so that jarring of the cage would not operate the lever. The lever was considered adequately placed if there were four or five operations per hour in the absence of food rewards.

To determine the optimum height of the lever, powdered graphite was sprinkled on the floor of a box lined with white paper. The region of the wall showing the most footprints would then be the area of most probable contact with the lever. The Swiss mice were observed to leave a band of prints between 2 and 4 inches from the floor with the greatest density near 3 inches. Accordingly this was chosen as the initial height for placing the lever. After the mouse had become conditioned to a reasonable rate of response the lever was lowered ½ inch to enable the animals to sit on their haunches and thereby perform better for a longer period. Even lower placement was necessary for optimum performance by the very obese mice. The pellets (0.04 g.) of a commercial feed were delivered by an

automatic feeder preset to furnish one pellet after a selected number of lever movements. The only disturbance occurred when the feeder was reloaded or the mouse weighed. The box was soundproofed and critical relay clicks inside were masked. That the method of conditioned response did not decrease the amount of food intake compared with free-feeding conditions was demonstrated in preliminary studies. At the time of writing, additional experiments were in progress in order to determine whether the "temporal distribution of the feeding behavior" was similar under the two conditions.

In addition to the expected greater overall rate of feeding for the hyperphagic mice there was a definite change in the daily pattern. In normal mice the approximately 24 hour cycle was characterized by an initial period of rapid feeding followed by a phase in which the rate was slower. No cycling was apparent in the obese mice until the weights reached a plateau at which point 24 hour cycles began to appear. The fact that the rates of the normal mice during the rapid feeding period were similar to the continuous rates for the hyperphagic mice suggested that the normal appetite is curbed by some mechanism resulting from satiety. The record reveals that even hyperphagic mice do not eat all the time.

Mayer (*Physiol. Rev.* **33,** *472 (1953)*) has previously stated that it is possible to produce only limited obesity by altering the composition or amount of experimental diets in the absence of a genetic predisposition. The method of operant conditioning and automatic feeding should be useful in exploring the factors which might inhibit the appetite of hyperphagic animals. It may possibly furnish some information which could be applied to the control of human obesity.

TRANS-ACIDS AS ESSENTIAL FATTY ACID ANTAGONISTS

The possibility of finding competitive inhibitors to the essential fatty acids (EFA) has occurred to many workers interested in fat metabolism. Thus, such inhibitors might be used to prevent certain types of rapid or abnormal growth as in tumors (I. Smedley-MacLean and L. C. A. Nunn, *Biochem. J.*, **35,** *983 (1941)*), or might be formed during hydrogenation of vegetable oils to the detriment of the users of these products (H. M. Sinclair, *Lancet* **270,** *381 (1956)*). However, the search for this type of inhibition has not been rewarding. No enzyme in which the essential fatty acids have been shown to play a definite role has been obtained from animal sources; attempts to produce inhibition in the usual test animals are complicated by their relatively large stores of essential fatty acids and their ability to alter or destroy the postulated inhibitors.

The logical compounds for trial as inhibitors would be those fatty acids possessing multiple double bond systems as do the essential fatty acids, but not in the 1:4 cis configuration. Such acids would be those containing conjugated double bonds or double bonds in the 1:4 trans arrangement. R. T. Holman (*Proc. Soc. Exp. Biol. Med.* **76,** *100 (1951)*) and Homan and S. I. Greenberg (*Arch. Biochem. Biophys.* **49,** *49 (1954)*), reported that the supplementation of a fat-free diet with conjugated "linoleic" acid resulted in lower weight gains and greater deficiency symptoms in rats than in control animals with no supplementation. D. Melnick and H. J. Deuel, Jr. (*J. Am. Oil. Chem. Soc.* **31,** *63 (1954)*; *Nutrition Reviews* **13,** *53 (1955)*), on the other hand, reported that although some of the trans positional isomers of oleic acid could not replace biotin for *L. arabinosus* and showed some toxicity, they were not inhibitors of oleic acid activity. Similarly these authors

found no evidence for EFA inhibition for rats from hydrogenated oils.

Recently, Holman and E. Aaes-Jorgensen (*Proc. Soc. Exp. Biol. Med.* **93,** *175 (1956)*) tested the effect of trans isomers of linoleic acid upon the development of EFA deficiency in rats. Male rats which had been on a fat-free diet containing 1 per cent each of cholesterol and hydrogenated coconut oil to accelerate the onset of deficiency symptoms were, after three months, divided into nine groups receiving the following supplements: ethyl elaidate, ethyl linolelaidate (with or without linseed oil), methyl cis, trans linoleate (with or without linseed oil), methyl linolenate, ethyl linoleate, linseed oil or no supplements. After three weeks of the supplements (0.1 g. per day), the rats were killed and the severity of the deficiency state, if any, was assessed by examination for skin symptoms, weight gain, carcass fatty acids, condition of spermatogenic tissue and deposition of dietary trans acids. Since each group contained only two rats, differences had to be very marked to be significant. The lowest weight gains were recorded with the cis, trans linoleate and the linolelaidate supplements although the other trans acid, ethyl elaidate, produced a weight increase somewhat greater than that for the fat-free diet. Linseed oil counteracted the effect of the trans dienes and gave weight gains similar to those for the other supplements.

The trans diene acids also produced the most severe skin symptoms, although in this case only ethyl linoleate was very effective in alleviating the condition. The other symptoms were of doubtful significance for this experiment since variations within groups were usually as great as those between groups. However, deposition of carcass fatty acids and recovery from testicular degeneration appeared to follow the same order as the other symptoms.

It is difficult to say, on the basis of these experiments, whether the trans acids indeed acted as competitive inhibitors of EFA action. That trans monoenoic esters from hydrogenation of triolein do not, was recently confirmed by R. B. Alfin-Slater *et al.* (*Ibid.* **95,** *521 (1957)*) in very similar experiments. However, it was not to be expected that the trans monoenoic acids would act in this manner, and the cis, trans and trans, trans dienoic esters apparently acted as inhibitors in that they increased the requirement of EFA and aggravated the deficiency symptoms resulting from a fat-deficient diet. In this connection it should be pointed out that the saturated fatty acids, cholesterol and perhaps several other substances are known to produce similar aggravation of the deficiency state, but are not likely to be antagonists.

Further advances in this field may well follow discovery of the mechanism of action of the essential fatty acids or at least development of an enzyme dependent on these substances for activity.

GLUCAGON EFFECT ON GASTRIC ACTIVITY

Glucagon, known as the pancreatic-hyperglycemic-glycogenolytic factor, HGF, has been the subject of several reviews dealing with its function. T. B. Van Itallie, (*New Engl. J. Med.* **254,** *794 (1956)*) has reviewed the physiologic and clinical aspects of glucagon and recent work has been summarized in *Nutrition Reviews* (**12,** *132 (1954)*; **13,** *325 (1955)*; **14,** *304, 309 (1956)*). The discovery of glucagon was made by C. P. Kimball and J. R. Murlin (*J. Biol. Chem.* **58,** *337 (1923)*) while determining the efficacy of various solvents and precipitants in the purification of insulin. J. F. Fodden and W. O. Read (*Endocrinology* **54,** *303 (1954)*) observed a depletion of the hyperglycemic action of the pancreas by the use of synthalin A, a material that is

toxic to pancreatic alpha cells. This evidence that the alpha cells are the source of glucagon has been supported by the observation of S. A. Bencosme, E. Liepa and S. S. Lazarus (*Proc. Soc. Exp. Biol. Med.* **90,** *387 (1955)*). They showed that extracts of the uncinate process of the dog pancreas, which is devoid of alpha cells, contained no hyperglycemic activity. The mechanism by which glucagon causes a rise in blood sugar is its glycogenolytic action on the liver, according to E. W. Sutherland and C. F. Cori (*J. Biol. Chem.* **188,** *531 (1951)*). R. M. Zollinger and E. H. Ellison (*Ann. Surg.* **142,** *709 (1955)*) proposed that peptic ulceration associated with gastric hyperacidity, which could not be controlled surgically or medicinally, might be the result of certain pancreatic islet cell tumors producing excessive amounts of glucagon. C. S. Hoar and J. R. Browning (*New Engl. J. Med.* **255,** *153 (1956)*) demonstrated that decreases in blood pepsinogen levels correlate directly with induced decreases in gastric peptic activity.

A. S. Earle, G. F. Cahill and Hoar (*Ann. Surg.* **146,** *124 (1957)*), by employing the determination of blood pepsinogen levels, endeavored to determine whether or not glucagon played a role in gastric secretory activity. Mongrel dogs weighing 8 to 15 kg. were fed a standard ration until 18 hours before the beginning of each experiment. Amorphous glucagon was administered intravenously during pentobarbital anaesthesia and, femoral vein blood was analyzed at intervals for glucose and pepsinogen levels. The rapid injection of 200 micrograms of glucagon resulted in glucose values between 184 and 232 mg. per cent, reached between 15 and 30 minutes after injection. The simultaneous decrease in blood pepsinogen level from 300 to 260 units, though probably significant, indicated the necessity for testing the effects of glucagon administered by continuous drip.

Infusions lasting from two to four hours, with dose rates ranging between 2.5 and 3.3 micrograms per minute, resulted in significant decreases in blood pepsinogen levels during the induced hyperglycemia. The average control level for blood pepsinogen of 420 units fell to 320 and 250 units after one half and two hours of glucagon infusion, respectively. During the hour after the completion of infusion the blood sugar values returned to normal, but the blood pepsinogen levels remained low. The fact that no significant alteration of normal blood glucose or pepsinogen level occurred during anesthesia alone eliminated the possibility that pentobarbital was responsible for depressing gastric activity. In order to learn whether the vagi influenced gastric activity after glucagon, the infusion was repeated in animals that had undergone vagotomies one week before. Although the blood pepsinogen level dropped following vagisection, a further significant drop was observed following the infusion of glucagon over a two-hour period. Intravenous infusion of glucose was used in an additional experiment to determine whether the hyperglycemia was responsible for the drop in pepsinogen level. The animals showed significant decreases in blood pepsinogen levels comparable to those seen following glucagon injection.

The injection of glucagon into human subjects showed results essentially similar to those seen in the experimental animals. The rapid injection of 0.8 mg. elicited a moderate rise in blood glucose (74 mg. per cent to 134 mg. per cent) and a decrease in blood pepsinogen from 360 units per ml. to 335 units per ml. Subjects 2 and 3 received infusions over a two-hour period and the gastric acid collections were evaluated for hourly volume and acidity. Subject 2, a normal volunteer, was given 1.75 mg. of glucagon during two hours and subject 3 with duodenal ulcer, associated hyperacidity, and elevated blood pepsinogen was given 2.5 mg. of glucagon during two hours. The blood pepsinogen levels of both

subjects dropped more than 50 units and the milliequivalents of total acid decreased.

The suggestion that the pancreas contains some principle which is active in the production of ulcers is supported by earlier experiments of L. R. Dragstedt (*Arch. Surg.* **44,** *338 (1942)*), in which he observed that animals undergoing complete loss of pancreatic juice by means of an external fistula developed ulcers in 100 per cent of the cases, whereas pancreatectomy gave almost complete immunity to ulcerogenesis. E. J. Poth and S. M. Fromm (*Gastroenterology* **16,** *490 (1950)*) observed peptic ulcer formation in dogs receiving insulin and suggested that the glucagon contamination of the insulin was responsible for the production of ulcers. However, no increase in the number of ulcers was seen following the addition of glucagon to the insulin injection. They then postulated that hypoglycemia was producing the effect. This theory received support in the report of H. S. Janowitz and B. B. Crohn (*Gastroenterology* **17,** *578 (1951)*) who observed duodenal ulcer formation associated with hyperinsulinism due to a beta cell adenoma.

A. J. Stunkard, T. B. Van Itallie and B. B. Reis (*Proc. Soc. Exp. Biol. Med.* **89,** *258 (1955)*) noted that injection of glucagon in human volunteers abolished hunger contractions. In contradistinction to the experiments of Zollinger and Ellison, these findings suggest that glucagon, rather than stimulating it, depresses gastric secretory activity. Further study is needed to determine the identity and define the role of the pancreatic ulcerogenic factor. Patients with intractable ulcers should be studied carefully for evidence of islet cell tumors. The histological and histochemical characteristics of any pancreatic tumors associated with peptic ulceration should be evaluated carefully.

NOTES

Letter to the Editor

Sir:

We are grateful for the informative review (*Nutrition Reviews* **15,** *198 (1957)*) of our article, "Absorption and Metabolism of Calcium in Human Beings, Studied with Calcium-45" (*Ann. N. Y. Acad. Sci.* **64,** *314 (1956)*), but we would like to point out several errors made by the reviewer.

We stated "the specific activity of calcium in the serum had not *leveled* off even two months after injection," rather than that it "did not taper off."

We did not conclude "that the major route of excretion in the human, however, was found to be via the urine." Our data indicate that endogenous calcium is partitioned approximately equally between urine and feces, with neither route dominant.

An error in a decimal point in your review suggests that the accretion rate of calcium in the young adults we studied was 75 g. Ca/day, instead of the correct 0.75 g./day.

Felix Bronner
Robert S. Harris

American Board of Nutrition

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition, during the week of April 13–19, 1958, at Philadelphia, Pennsylvania. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March. 1. Application forms may be obtained from the Secretary, Otto A. Bessey, Environmental Protection Research Division, Quartermaster Research and Development Center, Natick, Massachusetts.

Third Inter-African Conference on Nutrition

The Proceedings of the Second Inter-African Conference on Nutrition (*Nutrition Rev.* **13,** *287 (1955)*) held in Gambia in 1952 provided some insight into the nutritional problems of the area south of the Sahara, the research going on, and the experience of people intimately associated with the area. The preliminary report of the Third Conference held in Luanda, Angola, has now appeared (Reports of the Third Inter-African Conference on Nutrition. London Joint Secretariat Publications & Information Officer London). This represents a change in publishing policy for the report consists essentially of the introductory and closing speeches and the recommendations of the conference. Presumably the scientific papers will be published later. Whether this is a useful change of policy may be doubted. While speeches are necessary for any conference, they are the frosting on the cake which contributes little to the nutritional value of the contents. In view of the bulk of literature now appearing, the publication of a volume which essentially expresses the good intentions of the member governments is a disappointment. Similarly, it may be doubted whether lists of recommendations will be articularly productive.

What to do with conference proceedings is a continuing problem. It should be recognized that much of the material presented at such meetings is in the nature of progress reports rather than definitive papers. Thus, long delays in publication seriously detract from the value of the proceedings. Mimeographed copies of papers might be the most useful method with more formal publication restricted to papers which clearly warrant permanent recording. As Dr. F. W. Fox remarks in his opening address, "These conferences are not ends in themselves but successive steps in the campaign to banish the scourge of malnutrition from Africa."

Dr. Fox also has some other stimulating remarks to make with regard to present day nutrition research and practice and where it may lead. For example: "Must we not, then, guard against being carried away by the fascinating by-ways of biochemistry, constantly relating our findings to the main strategy, which is the contribution they can make towards planning for improved nutrition?" He points out that careful studies related to pellagra have contributed much to the knowledge of metabolism, but that "the virtual disappearance of this disease in the Southern States of the U. S. A. was not due to this new knowledge or its application through the distribution of nicotinic acid, or its use in the fortification of corn meal, but by all around improvement of living conditions. If and when kwashiorkor is banished from African history, it is likely to have a similar story to tell."

He points also to the fact that the "desired marriage" between agriculture and nutrition "has not advanced beyond the stage of a mild flirtation" although the greatest hope for improving nutrition must lie in having sufficient knowledge so that the "right crops can be grown in the right places in the right way and then adequately stored." Much more should be known about the indigenous edible plants in terms of use, nutritional value, production yields and methods, and similar considerations. At the same time new foods can often be introduced. Not long ago fish were considered a fundamental aversion of various South African tribes, but its popularity is now rapidly increasing. The "wisdom, inherent knowledge and experience of those who lead primitive lives" must be respected. He also notes that the intractability of the primitive African is often due to "his preoccupation with the desire to purchase leisure. . . .When planning for better nutrition this outlook has economic aspects that are most exasperating to the Western mind, which are not made any the easier by the sneaking feeling that here at least the African may be wiser than we are!"

Fox concludes with an emphasis upon the

so-called "population problem." W. H. Forbes (*Fed. Proc.* **11,** *667 (1952)*) emphasized that the ratio food resources/population (F/P) "determines the whole nature of a civilization." Although FAO and other organizations have done considerable to increase F, and means are available to do much more, no comparable success has been associated with stabilizing P. Until this is accomplished, it would seem that the world will move toward what Forbes called a state of "stable poverty." "Considering the usual African rural community, it would usually be possible to obtain a startling increase in F. . . . However, it would be at least as easy, perhaps easier, to decrease the high rate of mortality during childhood, which would rapidly increase P. Since our resources of personnel and money are limited, I have always argued that we must first concentrate on stabilizing and improving the agricultural foundations in such circumstances, rather than promote medical, maternity, and child welfare services. It was therefore stimulating to find Platt take the opposite view, partly because . . . those who work for the welfare of mothers and children . . . are strategically placed to give effective advice on family planning. I would like to be convinced that an education policy of this type can be effectively implemented in our African setting, because it is population size rather than food resources that will in the long run determine the level of nutrition."

Recent Nutrition Books

Indian Council of Medical Research. The Nutrition Research Laboratories (Coonoor, South India). Annual Report for 1955–56. Pp. 47.

Malnutrition and Nutrition Activities in Japan. Joint FAO/WHO Nutrition Committee for the South and East Asia Fourth Meeting, Tokyo, Japan. September–October, 1956. Ministry of Health and Welfare, Japanese Government, Tokyo, Japan. Pp. 184.

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The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

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